Comparative floral structure and development of Nitrariaceae (Sapindales) and systematic implications

Bachelier, J B ; Endress, P K ; Ronse de Craene, L

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Comparative floral structure and development of Nitrariaceae (Sapindales) and systematic implications

Julien B. Bachelier, Peter K. Endress and
Louis P. Ronse De Craene

8.1 Introduction

For the last 20 years, the development and improvement of molecular methods, based mostly on the comparison of DNA sequences, have been increasingly successful in reconstructing the phylogenetic tree of plants at all hierarchical levels. Consequently, they have contributed greatly to the recent improvement of angiosperm systematics. In addition, they have shown that earlier classifications, based mostly on plant vegetative and reproductive structures, had sometimes been misled by homoplastic characters, and a number of orders and families have had to be newly circumscribed or even newly established (e.g. APG, 1998, 2003, 2009; Stevens, 2001 onwards). These new results provide a novel basis for comparative structural studies to characterize the newly recognized clades and to evaluate clades that have only limited molecular support. However, because such comparative studies are time-consuming and the systematic classification has different hierarchical levels, they can only be done in a stepwise fashion (for eudicots, e.g. Matthews and Endress, 2002, 2004, 2005a, b, 2006, 2008; von Balthazar et al., 2004; Schönenberger and Grenhagen, 2005; Endress and Matthews, 2006; Ronse De

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Craene and Haston, 2006; von Balthazar et al., 2006; Bachelier and Endress, 2008, 2009; Janka et al., 2008; Schönenberger, 2009; von Balthazar and Schönenberger, 2009; Schönenberger et al., 2010).

As part of such a comparative approach, we studied the floral structure of Nitrariaceae, a small family which has been recently reclassified in Sapindales (APG, 2009). Nitrariaceae comprise four genera and around 15 species (Stevens, 2001 onwards; APG, 2009). They are native to arid and semi-arid regions of the Old World and are small to medium-sized shrubs (*Nitraria*; Engler, 1896a, b; Bobrov, 1965; Noble and Whalley, 1978), perennial herbs (*Peganum* and *Malacocarpus*; Engler, 1896a, 1931; El Hadidi, 1975) or small annual herbs of only a few centimetres height (*Tetradiclis*; Engler, 1896b, 1931; Hamzaoğlu et al., 2005). In earlier classifications, the position and the mutual affinities of these genera varied tremendously, depending on the weight an author gave either to their vegetative or their reproductive features (Takhtajan, 1969, 1980, 1983, 2009; El Hadidi, 1975; Dahlgren, 1980; Cronquist, 1981, 1988; see Sheahan and Chase, 1996 for a detailed review of classifications). Because none of the traditional classifications was entirely satisfactory, however, most authors followed Engler's influential work (1896a, b, 1931) and *Nitraria*, *Tetradiclis* and *Peganum* (including *Malacocarpus*) remained for a long time in their own subfamilies in Zygophyllaceae (Nitrarioideae, Tetradiclidoideae and Peganoideae; for more details of the history of classification, see Sheahan and Chase, 1996).

Molecular phylogenetic studies have shown that the earlier Zygophyllaceae *sensu* Engler (1896a, b, 1931) comprised a cluster of unrelated genera. They first showed that Nitrariaceae were more closely related to Sapindales of malvids than to the remainder of Zygophyllaceae, which are now in Zygophyllales of fabids (Fig 8.1A; Fernando et al, 1995; Gadek et al., 1996; Sheahan and Chase, 1996; Bakker et al., 1998; Savolainen et al., 2000; Muellner et al., 2007; Wang et al., 2009; Worberg et al., 2009). These studies also showed that *Nitraria*, *Peganum*, *Malacocarpus* and *Tetradiclis* consistently form a clade, in which *Nitraria* is always sister to the other three, and *Tetradiclis* is sister to *Peganum* + *Malacocarpus* (Fig 8.1B; Sheahan and Chase, 1996; Savolainen et al., 2000; Muellner et al., 2007). However, the position of the Nitrariaceae with regard to the other families of Sapindales is not well resolved yet, and depends on the sampling size of the outgroup and the ingroup, as well as on the DNA regions used. For instance, if *Nitraria* and *Peganum* are taken separately, they appear as sister to the remainder of Sapindales in Fernando and Quinn (1995) and Worberg et al. (2009), but *Nitraria* appears as sister to the clade of Anacardiaceae and Burseraceae in Wang et al. (2009). If *Nitraria* and *Peganum* (including *Malacocarpus*) are taken together, they appear as sister to the remainder of Sapindales (Gadek et al., 1996; Bakker et al., 1998). In contrast, when *Tetradiclis* is added, Nitrariaceae are nested within Sapindales, but they are either sister to

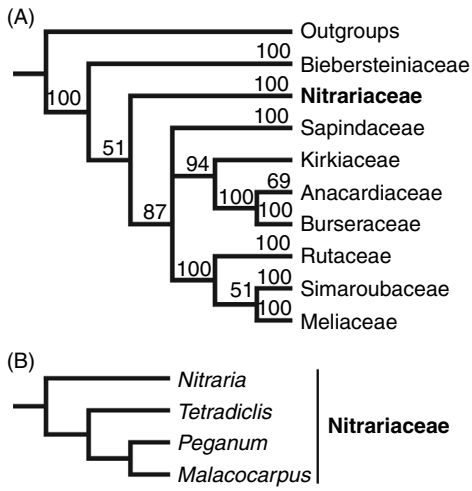

Fig 8.1 (A) Phylogeny of Sapindales, based on *rbc*L sequences (Bayesian posterior probabilities indicated above the branches; simplified from Muellner et al., 2007). (B) Phylogeny of Nitrariaceae, based on several studies using molecular and non-molecular data (Sheahan and Chase, 1996; Savolainen et al., 2000; Muellner et al., 2007).

the clade of Anacardiaceae + Burseraceae (Savolainen et al., 2000), or appear as the second basal most sapindalean family (Muellner et al., 2007).

It is now clear that the similarities in vegetative gross morphology shared by the earlier Zygophyllaceae have only limited phylogenetic significance and are just adaptive convergences due to their similar extreme habitats. Earlier comparative studies also indicated that these four genera, especially *Nitraria* and also *Peganum* (with *Malacocarpus*), were more distinct from the remainder of Zygophyllaceae than previously suggested (for vegetative anatomy, see Sheahan and Chase, 1993; for palynology, see Erdtman, 1952; Agababyan, 1964; Perveen and Qaiser, 2006; for embryology, see Mauritzon, 1934; Souèges, 1953; Kapil and Ahluwalia, 1963; Kamelina, 1985, 1994; Li and Tu, 1994; for floral structure, see Baillon, 1873; Nair and Nathawat, 1958; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996; for chemistry, see Hussein et al., 2009). In contrast to most of the current Zygophyllaceae, which have pentamerous, isomerous and obdiplostemonous flowers, the flowers in *Tetradiclis* are tetramerous, isomerous and haplostemonous, while in *Nitraria* and *Peganum* (+ *Malacocarpus*), the flowers have a pentamerous perianth and androecium, conspicuous antepetalous stamen pairs and a tricarpellate gynoecium (Payer, 1857; Baillon, 1873; Engler, 1896a, b, 1931; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). Nair and Nathawat (1958) mentioned that *Peganum* and *Nitraria* have a similar vascular

anatomy and a distinct androecium, with five groups of three antesepalous stamens, but some comparative developmental studies of the unusual androecium of *Nitraria* and *Peganum* suggested that the pairs were not homologous (Payer, 1857; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). In *Nitraria*, each pair belongs to a triplet of antesepalous stamens and is thus derived from a haplostemonous pattern (Payer, 1857; Ronse De Craene and Smets, 1991) whereas, in *Peganum*, each pair has been interpreted to be formed by duplication of the antepetalous stamen primordium and, therefore, derived from an obdiplostemonous pattern (Payer, 1857; Eckert, 1966; Ronse De Craene and Smets, 1996; Ronse De Craene et al., 1996).

Except for the opinion that *Nitraria* and *Peganum* + *Malacocarpus* may not belong to the Zygophyllaceae, it has neither been suggested that these genera are closely related, nor that they would be part of the Sapindales (except for Cronquist, 1981, who, in any case, placed the entire Zygophyllaceae in Sapindales). The new light shed on the relationships between these genera and their affinities by molecular studies provides new ground to perform a comparative study of their flower structure and development. This enables us to re-evaluate the unusual androecium of *Nitraria* and *Peganum* and their affinities with *Tetradiclis*, as well as with other sapindalean families.

8.2 Material and methods

The following material has been studied:

> *Nitraria retusa*: P. Endress, s.n., 07 May 1971, Algeria; L. Ronse De Craene 43LS, Senegal; 302LT, Tunisia.
>
> *Peganum harmala*: P. Endress, s.n., 01 May 1971, Algeria.
>
> *Tetradiclis tenella:* G. Woronow and P. Popow, s.n., 18 May 1971, Azerbaijan [Z-ZT]; J. Bornmüller 640, Iraq [Z-ZT]; P. Sintenis 1537, Turkmenistan [Z-ZT].

The material was studied using light microscopy (LM) and scanning electron microscopy (SEM). Fresh flowers of *Nitraria* and *Peganum* were collected and stored in 70% ethanol, while material of *Tetradiclis* was taken from herbarium collections. The dry flower buds and anthetic flowers of *Tetradiclis* were soaked in dioctyl sodium sulfosuccinate solution for three days and stored in 70% ethanol.

For LM investigations, part of the material of *Peganum* and *Nitraria* was prepared according to the standard paraplast embedding protocol, whereas the material of *Tetradiclis* plus additional material of *Peganum* and *Nitraria* were embedded in plastic using Kulzer's Technovit 7100 (2-hydroxyethyl

methacrylate) following a protocol adapted from Igersheim (1993) and Igersheim and Cichocki (1996). Serial microtome sections were made at 5, 7, 10 or 15 μm. Sections in paraplast were stained with safranin and Astrablue and mounted in Eukitt, whereas sections in plastic were stained with ruthenium red and toluidine blue and mounted in Histomount (protocol adapted from Weber and Igersheim, 1994).

For SEM investigations, specimens were stained with 2% osmium tetroxide, dehydrated in ethanol and acetone, critical-point dried and sputter-coated with gold, and studied at 20 kV with a Hitachi S-4000 scanning electron microscope. The liquid-fixed material and the permanent slides of serial microtome sections are deposited at the Institute of Systematic Botany of the University of Zurich (Z).

8.2.1 General comments on illustrations

Figures 8.2 to 8.8 represent structural analyses of the genera studied. In all drawings, the morphological surfaces are drawn with thick continuous lines; pollen tube transmitting tracts are shaded dark grey (only in anthetic gynoecia); nectaries are shaded light grey. In schematic longitudinal sections of gynoecia, (A) shows a median section of two carpels projected onto the drawing plane because of the odd number of carpels, while (A′) shows a longitudinal section through the middle of one carpel and the area between the two other carpels. Outlines of parts outside the median plane are drawn with thick broken lines; postgenitally fused surfaces are hatched; embryo sacs are drawn with thin continuous lines. In transverse microtome section series, sections are ordered from top, downwards; postgenitally fused surfaces and pollen sacs are drawn with thick broken lines; vascular bundles are drawn with thin continuous lines.

8.2.2 Glossary

Angiospermy type 3 Carpels closed by postgenital fusion at the entire periphery, but with an open canal in the inner angle of the ventral slit (Endress and Igersheim, 2000).
Angiospermy type 4 Carpels completely closed by postgenital fusion (Endress and Igersheim, 2000).
Antitropous Ovule, in which the direction of curvature is opposite to the direction of carpel closure (Fig 8.3B, see also Endress, 1994), more or less corresponding to epitropous (*sensu* Agardh, 1858).
PTTT Pollen tube transmitting tract.
Syntropous Ovule, in which the direction of curvature is the same as the direction of carpel closure (Fig 8.3A, see also Endress, 1994), more or less corresponding to apotropous (*sensu* Agardh, 1858).

8.3 Results

8.3.1 *Peganum harmala*

Morphology

The flowers are relatively large (2 to 4 cm across), morphologically bisexual and entomophilous. The floral organ whorls are pentamerous, except for the trimerous gynoecium (Figs 8.2–8.5). The androecium is seemingly two-whorled and obdiplostemonous; the antepetalous stamen whorl gives the impression of being the outer one and the antesepalous the inner one (Figs 8.2B–D, 8.5). In addition, the androecium is unusual because the outer whorl comprises five stamen pairs instead of five single stamens (Figs 8.2B–D, 8.5).

The free sepals are long and narrow and have one or two (rarely more) small basal lateral appendages (Figs 8.2, 8.4A). In bud, their aestivation varies during development (Figs 8.2, 8.4A; see Section 8.3.1, Development). The petals are elliptical and mucronate. They have a pronounced median dorsal rib (Figs 8.2A–D, 8.4B), and rarely two or three tips. Their aestivation is somewhat open at the base, and further up, it is contort. Postgenital coherence is formed between their overlapping parts by interdentation of their papillate epidermis and cuticular ornamentation, and by interlocking glandular hairs on the margins of the petal tips (Fig 8.4A). At anthesis, however, the perianth is wide open and the inner reproductive organs are entirely exposed.

All stamens are free and similar in shape (Fig 8.2B–D). The filament bases are broad and thick with conspicuously flattened margins (Fig 8.4C, D). They overlap slightly with each other and form a collar around the ovary. Further up, the filaments become narrower and more rounded, and the transition from filament to anther is constricted (Fig 8.4C, D). The anthers are sagittate and (slightly dorsally) basifixed (Fig 8.4C). The dorsal side of the thecae is slightly larger and much longer than the ventral one, and the anthers are thus slightly introrse and apiculate (Figs 8.2B, 8.4C, E). Each theca has a longitudinal dehiscence line, which encompasses its upper and lower shoulders (Fig 8.4C). Between the stamens and gynoecium base there is a lobed nectary disc with five conspicuous antepetalous depressions (Figs 8.2E, 8.3O).

The gynoecium is syncarpous and polysymmetric and has a superior ovary (Figs 8.2B–D, 8.3, 8.4F). However, because of the difference in merism of gynoecium and the outer whorls of organs, the flower is in some way monosymmetric (Fig 8.2). The gynoecium has a short and stout gynophore, a globose ovary with conspicuous dorsal bulges surrounding and hiding the base of a long and slender style (Figs 8.3A, 8.4F, G), which is narrower and more rounded at the base than further up (Fig 8.3A–G) and becomes triangular at mid-length (Figs 8.3B–D, 8.4F, H). The upper part of the gynoecium is apocarpous for a long distance, but with

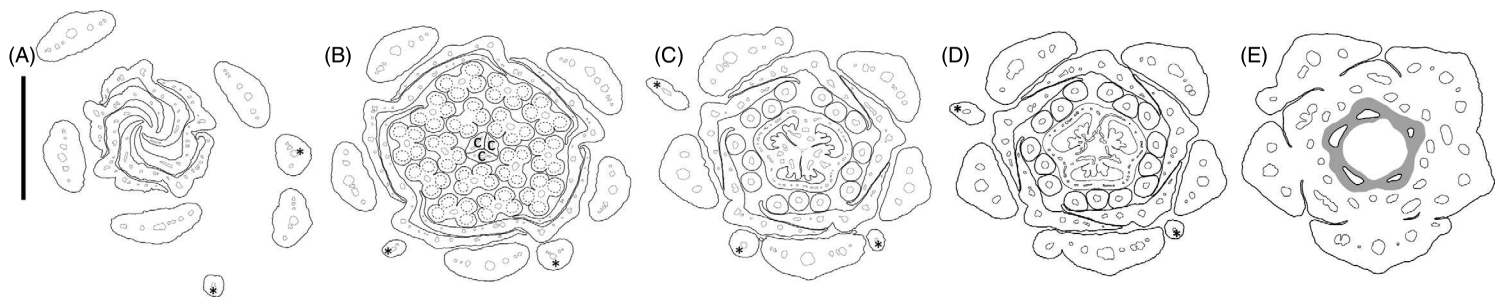

Fig 8.2 *Peganum harmala*. Floral bud, transverse section series. Sepal basal lateral appendages marked with asterisks [*]. (A) Distal zone, five sepals and five petals with contort aestivation. (B) Fifteen stamens surrounding three carpel tips [c], free but postgenitally united. (C) Stamen filament bases in five antesepalous triplets, transition from symplicate to synascioliate zone of the ovary at the level of the placentae. (D) Synascidiate zone of the ovary. (E) Floral base, with five antesepalous nectariferous depressions. Scale bar: (A)–(E) = 500 µm.

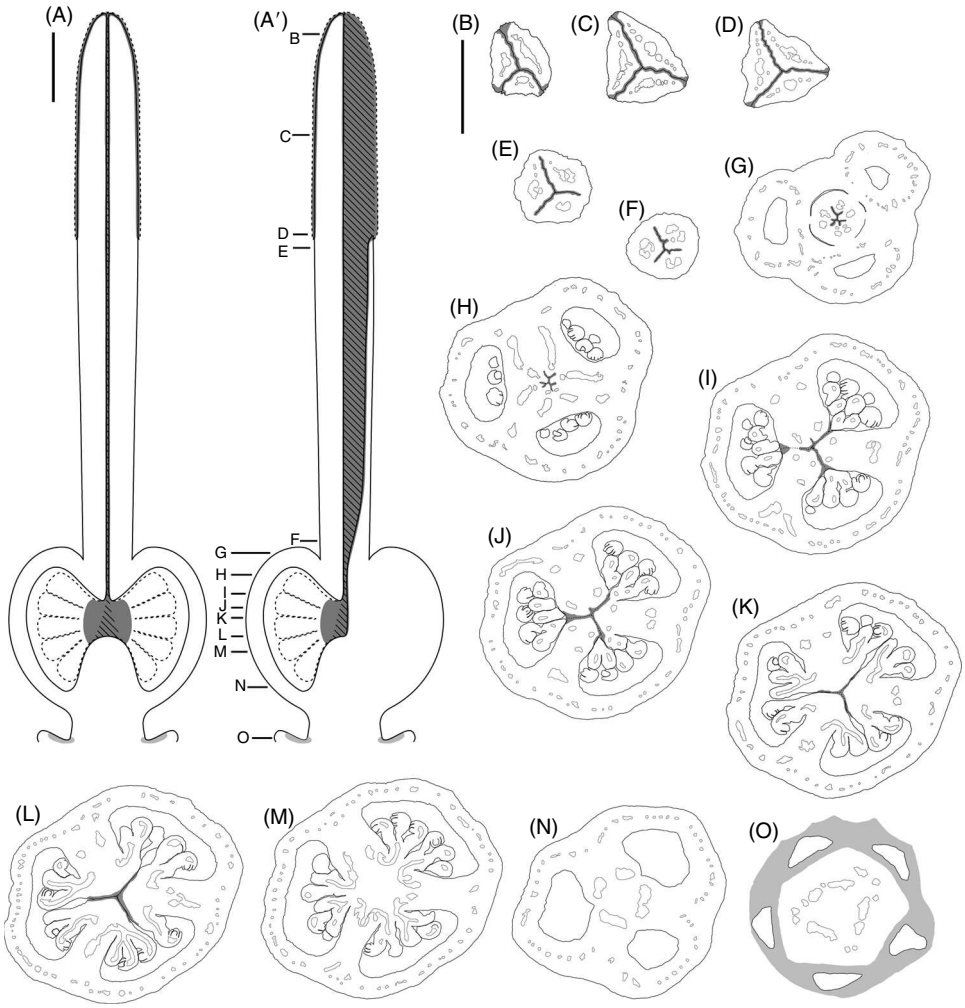

Fig 8.3 *Peganum harmala*. Anthetic gynoecium. (A) and (A') (see Section 8.2.1) schematic median longitudinal Section. (B)–(O) Transverse section series. (B)–(D) Apocarpous zone, carpel tips postgenitally united, stigmas decurrent along carpel margins. (E)–(L) Symplicate zone. (E)–(G) Style. (G–H) Apical septum. (K)–(M) Transition between symplicate and synascidiate zone, with axile lateral placentae protruding into the locules and bearing many ovules. (M), (N) Synascidiate zone. (O) Below the ovary, five antepetalous depressions surrounded by nectariferous tissue. Scale bars: = 1 mm.

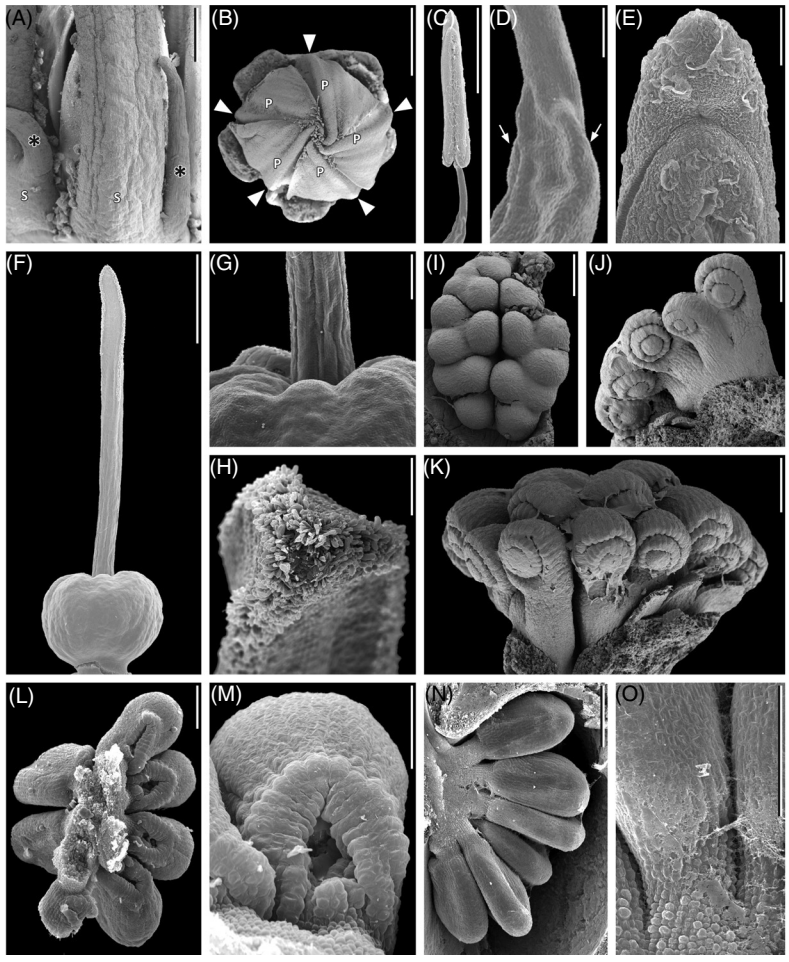

Fig 8.4 *Peganum harmala*. Floral organs. (A) Floral bud, lateral view. Sepal bases marked with [S] and basal lateral appendages with an asterisk [*], (B) Floral bud, older than (A), sepals removed, from above. Petal contort aestivation, with multicellular hairs on tips. Petals [P], dorsal median ridge indicated by white arrowheads. (C)–(E) Stamen. White arrows point to flattened margins of the filament. (C) Stamen, ventral view. (D) Close-up of filament base. (E) Close-up of apical beak. (F)–(H) Gynoecium. (F) Lateral view. (G) Close-up of style base and ovary dorsal bulges. (H) Close-up of three-angled stigmatic head from above, with three papillate stigmatic crests decurrent on edges. (I)–(O) Ovules. (I) Early stage of development, two collateral placentae, each with two vertical rows of ovule primordia. (J) Later in development, young ovules on branched placenta, with circular inner integument and hood-shaped outer integument. (K) Two collateral placentae bearing slightly older ovules. (L) Anthetic ovules borne on a single placenta. (M) Close-up, micropyle, with loosely closed exostome. (N) Anthetic placenta still in position, with smooth PTTT and dorsal sides of ovules plus funicles. (O) Close-up, papillate dorsal side of funicle covered with secretion. Scale bars: (C), (F) = 3 mm; (B) = 1 mm; (G), (H) = 500 μm; (A), (D), (E) = 300 μm; (L), (N) = 200 μm; (J), (K), (M), (O) = 100 μm, (I) = 50 μm.

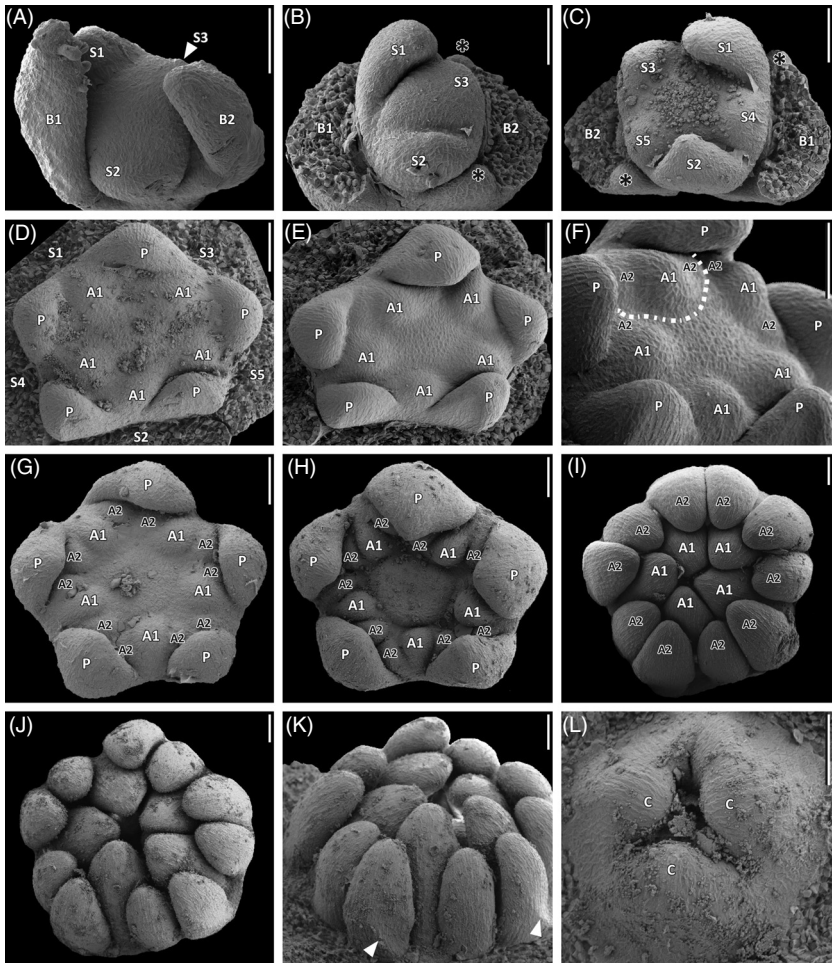

Fig 8.5 *Peganum harmala*. Flower buds, successive developmental stages. Sepals marked with S, petals with P, antesepalous stamens with A1, antepetalous stamens with A2, carpels with C. Orientation of buds with sepal S2 in median lower position. Prophyll and sepal initiation is counterclockwise in (A), (B) and (D), and clockwise in (C). (A) Two lateral prophylls [B1 and B2], and first and third sepals [S1 and S3] in anterior position, and second sepal [S2] posterior. (B) Prophylls B1 and B2 removed, basal appendages on prophylls marked with an asterisk [*]. (C) Initiation of lateral sepals [S4 and S5]. (D)–(H) Sepals removed. (D) Pentagonal inner part of young flower with five petal at the angles [P], and five antesepalous stamen primordia [A1]. (E), (F) Slightly older stage. Appearance of smaller antepetalous pairs of stamen primordia between the antesepalous ones [A2]. (E) From above. (F) Close-up, dotted line around a triplet of stamen primordia. (G) All stamens initiated, showing the differential development of A2 pairs relative to the neighbouring A1. (H) Syncarpous gynoecium primordium rising in the centre, compressed by the development of surrounding triplets of stamens. (I)–(K) Sepals and petals removed, showing differential growth of A2 pairs affected by the development of closest A1, and by the petals (white arrowheads). (L) Sepals, petals and stamens removed, syncarpous gynoecium with three carpels [C]. Scale bars: (A)–(L) = 50 μm.

the carpels postgenitally united; the carpels are flat in this zone (Figs 8.3A–D, 8.4H). Along the carpel margins stigmatic tissue is decurrent for a long distance as three wavy lines along the style (Figs 8.3A–D, 8.4F, H). Each of these three lines represents a partial external compitum (Figs 8.3A–D, 8.4H). The stigmatic lines exhibit unicellular, slightly secretory papillae (Figs 8.4H, 8.9A). The gynoecium is of angiospermy type 4 (Fig 8.3A–L). Below the stigmatic lines the carpels are congenitally united. The synascidiate zone extends up to the mid-length of the ovary (Fig 8.3A, M, N) and the symplicate zone up to mid-length of the style. The bulged zone of the ovary contains an apical septum (Fig 8.3A, E–L). Below the stigmas, the PTTTs differentiate along the ventral side of the carpels, and they are one cell layer thick and secretory (Figs 8.3A–L, 8.9A, B). They form an internal compitum, which is continuous with the external one and extends down to the transition zone between the symplicate and synascidiate zone of the ovary (Fig 8.3A–L). There, the carpel ventral sides are only loosely postgenitally united and a placenta differentiates on each of their margins (Fig 8.3A, K–M). There are two axile lateral placentae which develop into each locule, and each placenta bears *c.* 10 ovules (Figs 8.3J–M, 8.4I–N). The ovules are densely packed, but do not entirely fill the locule at anthesis (Fig 8.4N). Each ovule is anatropous, syntropous, crassinucellar and bitegmic (Figs 8.3J–M, 8.4J–M). The inner integument is 2–3 cell layers thick and is shorter than the outer one, which is 3–4 cell layers thick. The outer integument is hood-shaped, whereas the inner integument entirely surrounds the nucellus. Both integuments are thickened above the nucellus and form a micropyle, which faces the base of the funicle and comprises a circular endostome hidden by an exostome, which is not completely closed on the adaxial side (Fig 8.4L, M). At anthesis, the dorsal side of the funicle is papillate and covered with secretion (Fig 8.4N, O).

Development

Each flower develops in the axil of a subtending bract (pherophyll) and has two lateral prophylls which appear successively, opposite each other (Fig 8.5A–C). They are quickly followed by a first sepal primordium, which develops close to the larger (first) prophyll, and four additional sepal primordia which appear in a regular (clockwise or anticlockwise) spiral sequence (Fig 8.5A–C). The free sepals become valvate to slightly imbricate further up, depending on whether or not lateral basal appendages are present. When all sepals are initiated the floral apex becomes almost pentagonal, while five petal primordia appear more or less simultaneously, alternating with the sepals (see Figs 3–4 in Ronse De Craene et al., 1996). Initiation of the androecium quickly follows that of the corolla. The first whorl of five stamens alternates with the petals (Fig 8.5D–E, see Fig 6 in Ronse De Craene et al., 1996). Shortly thereafter, ten smaller stamen primordia appear more or less simultaneously in pairs between the antesepalous stamens (Fig 8.5E, F). However, separation soon appears to be deeper between the stamens of each antepetalous pair

than between the pair and the adjacent antesepalous stamens (Fig 8.5G). In addition, the difference in size and position between the stamen primordia of the two apparent whorls, as well as between the two stamens in each antepetalous position, strongly suggest that the androecium is primarily haplostemonous and that the antesepalous triplets develop centrifugally, with a first median and inner (or upper) stamen and two lateral and slightly outer (or lower) stamens (Figs 8.2C, D, 8.5F, G). In the floral centre, the syncarpous gynoecium first appears as a rounded bulge (Fig 8.5E), but while the surrounding pentamerous androecium enlarges, sometimes it becomes slightly pentagonal (Fig 8.5H; see also Fig 10 in Ronse De Craene et al., 1996). However, only three carpels clearly develop to anthesis (Fig 8.5L). The gynoecium was trimerous in all the material studied here. Petal development is not delayed, and they become longer than the sepals in bud, and thus protect the inner floral organs up to anthesis (Fig 8.2). The contort petal aestivation affects the growth of the outer stamens, which may reach different sizes at mid-development (Fig 8.5I–K). The outer antepetalous stamens are at first shorter than the antesepalous stamens, but become longer before anthesis.

At anthesis, the sepals and petals are fully expanded. The petals are no longer postgenitally connected. Nectaries are situated between the androecium and gynoecium, where they form five depressions in the petal radii (Fig 8.2E). After anthesis, the sepals are persistent. The petals and stamens fall off. The long style dries up distally, but also persists for some time and no abscission zone can be seen at anthesis.

Anatomy and histology

Each sepal has three main vascular traces at the base, but further up, there can be up to seven branches formed by successive lateral branching (Fig 8.2A–E). Sometimes, a main vascular trace and additional smaller ones are also present in the teeth at the base of the largest sepals. Petals and stamens have a single trace (Fig 8.2A–E). In petals there may be up to 14 vascular bundles (Fig 8.2A–D). In carpels, the dorsal vasculature forms a more or less continuous band along the entire length of the gynoecium (Figs 8.2B–D, 8.3B–N). The lateral vascular bundles are conspicuous only in the ovary septa. They differentiate from the dorsal vasculature at the base of the style (Fig 8.3F, G) and extend downward in the postgenitally united septa (Fig 8.3I, J). At the transition between the symplicate and synascidiate zone, each lateral bundle connects to a placenta (Fig 8.3J–M). Each placental bundle appears to be 'inverted' (xylem peripheral and phloem central) and splits into branches, which supply the ovules (Fig 8.3K–M). Only below the placentae, in the synascidiate zone, do the lateral bundles of adjacent carpels merge and form synlateral bundles, arranged around the floral centre, and extend downwards into the floral base (Fig 8.3N, O).

On the ventral side of the sepal bases, there is a dense carpet of hairs with a uniseriate multicellular stalk and a round, multiseriate and multicellular head.

Smaller, but similar, hairs are also found on the petal tips. No tanniferous cells or special mucilage cells (for term, see Matthews and Endress, 2006) have been observed in buds or anthetic gynoecia. During development, the mesophyll becomes lacunar in all organs.

8.3.2 *Nitraria retusa*

Morphology

The flowers are relatively small (*c.* 1 cm across), morphologically bisexual and entomophilous. The floral organ whorls are pentamerous, except for the trimerous (sometimes dimerous) gynoecium (Figs 8.6–8.8). The androecium is seemingly two-whorled and obdiplostemonous; the antepetalous stamen whorl gives the impression of being the outer one and the antesepalous the inner one. However, the androecium is unusual because the outer whorl appears to comprise five stamen pairs instead of only five single stamens (Figs 8.6A–D, 8.8D, E).

A difference in size of the sepals indicates that they are initiated in a spiral sequence. They are free in early stages of development but later their expanded bases become united. Their free tips are acute and become contiguous. They are valvate or may even be slightly overlapping (Fig 8.8A). The petals are free for their entire length (Fig 8.6). They have a narrow base, but become larger further up, with their margins folded inwards around a pair of antepetalous stamens. Their acute tips are strongly bent inwards around the thecae of adjacent antesepalous stamens (Fig 8.6B). The petals are thus hood-shaped. Their aestivation is shortly open at the base and induplicate-valvate further up (Figs 8.6, 8.8B, C). The contiguous margins are covered with interlocking hairs and are postgenitally coherent (Figs 8.6B–D, 8.8C). In young buds, when the floral base and sepal bases enlarge, the aestivation of the sepal free tips becomes open and the corolla takes over the protection of the inner organs up to anthesis (Figs 8.6A–D, 8.8A–C). In older buds, however, the corolla is not fully closed in the floral centre and the carpel tips are exposed before the flower opens (Figs 8.6A, B, 8.8D). It is unclear whether these flowers are bisexual and strongly protogynous, or functionally female. At anthesis, the calyx is wide open, but not the corolla because the petals remain erect, and the inner reproductive organs remain partly hidden.

The stamens are free (Fig 8.8E). In our material the ten antepetalous stamens are shorter and smaller than the five antesepalous ones (Figs 8.6A–C, 8.8E). The filaments are broad and irregularly thickened at the base, but become narrower and rounder further up. The transition from filament to anther is constricted (Figs 8.6A–D, 8.8E–G). The anthers are X-shaped and (slightly dorsally) basifixed, and versatile at anthesis. The dorsal side of the thecae is slightly larger and longer than the ventral one, and the anthers are almost latrorse (Figs 8.6B, C, 8.8F, G). Each theca has a longitudinal dehiscence line, which encompasses its upper and lower

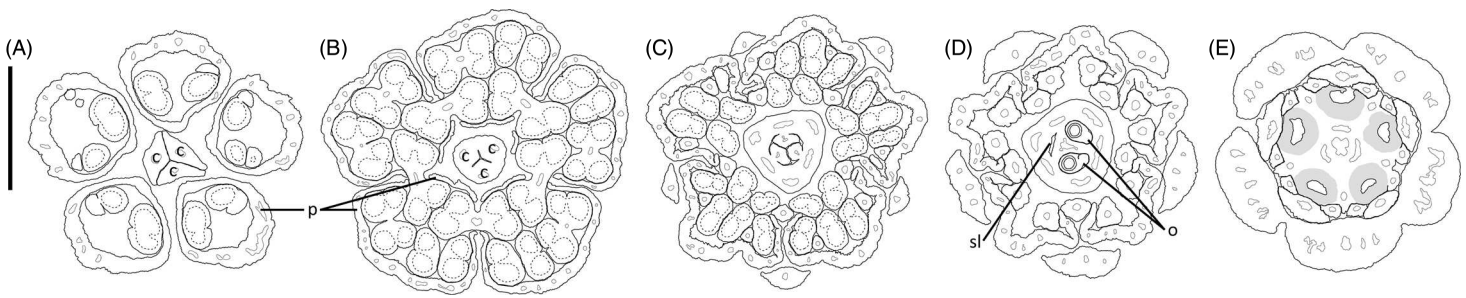

Fig 8.6 *Nitraria retusa*. Floral bud, transverse section series. (A) Distal zone, five petals [p] with valvate aestivation and tips bent inwards surrounding postgenitally united carpel tips [c]. (B) Fifteen stamens surrounding the style. (C) Ovary at the level of the placentae. (D) Ovary, with two fertile locules, each with one ovule [o], and one sterile, empty locule [sl]. (E) Floral base, with five antesepalous depressions (nectaries). Scale bars (A)–(E) = 1 mm.

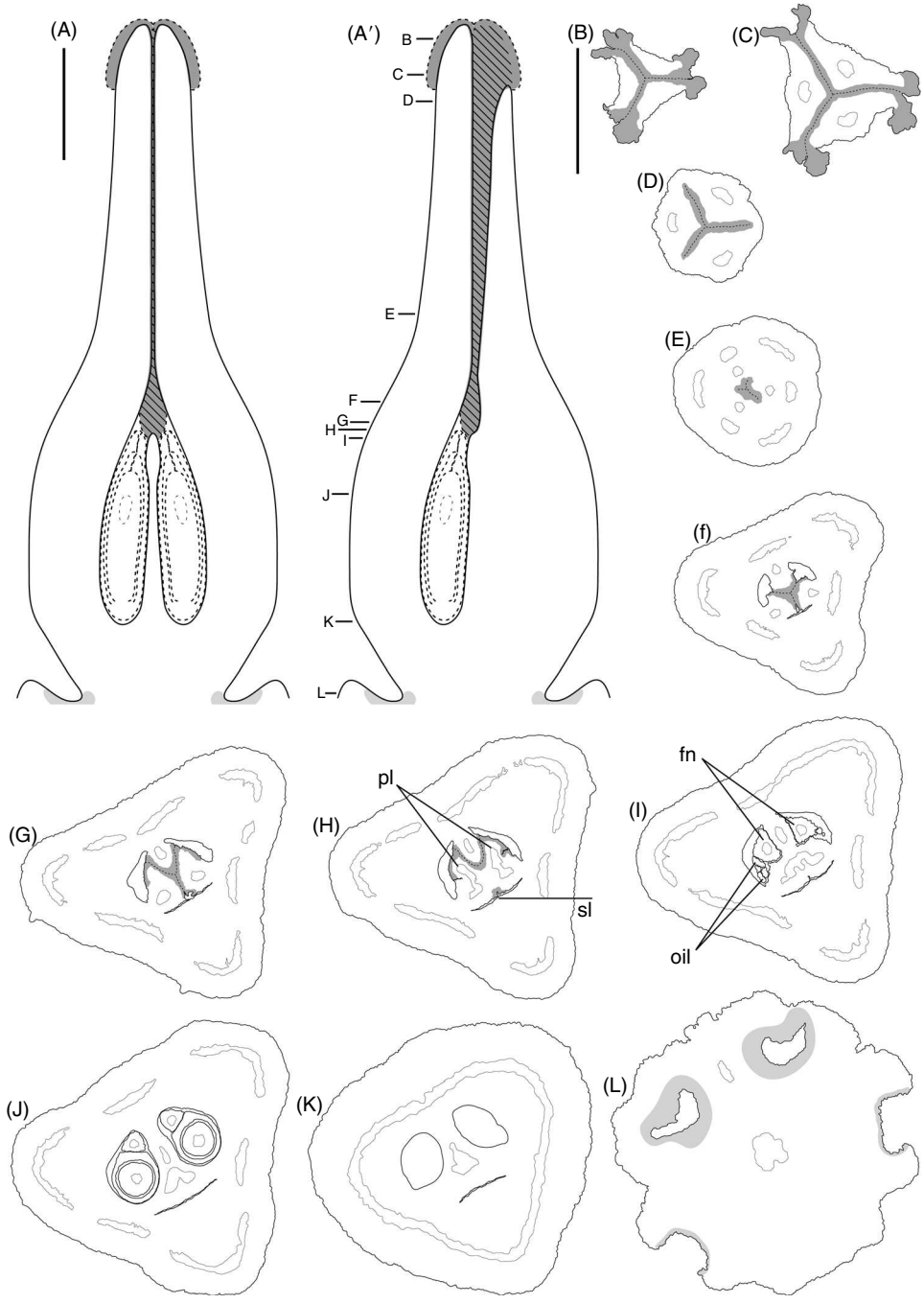

Fig 8.7 *Nitraria retusa*. Anthetic gynoecium. (A) and (A') (see section 8.2.1) Schematic median longitudinal section. (B)–(L) Transverse section series. (B), (C) Apocarpous zone, carpel tips postgenitally united, stigmas decurrent along carpel margins. (D)–(G) Symplicate zone, style. (H) Transition between symplicate and synascidiate zone, with two fertile locules, each having one apical axile lateral placenta [pl], and one sterile locule [sl]. (I)–(K) (Synascidiate zone.) (I) Showing funicles [fn] and contiguous outer integument lobes of the ovules [oil]. (L) Below the ovary, five (four visible) antepetalous depressions (nectaries). Scale bars: = 2 mm.

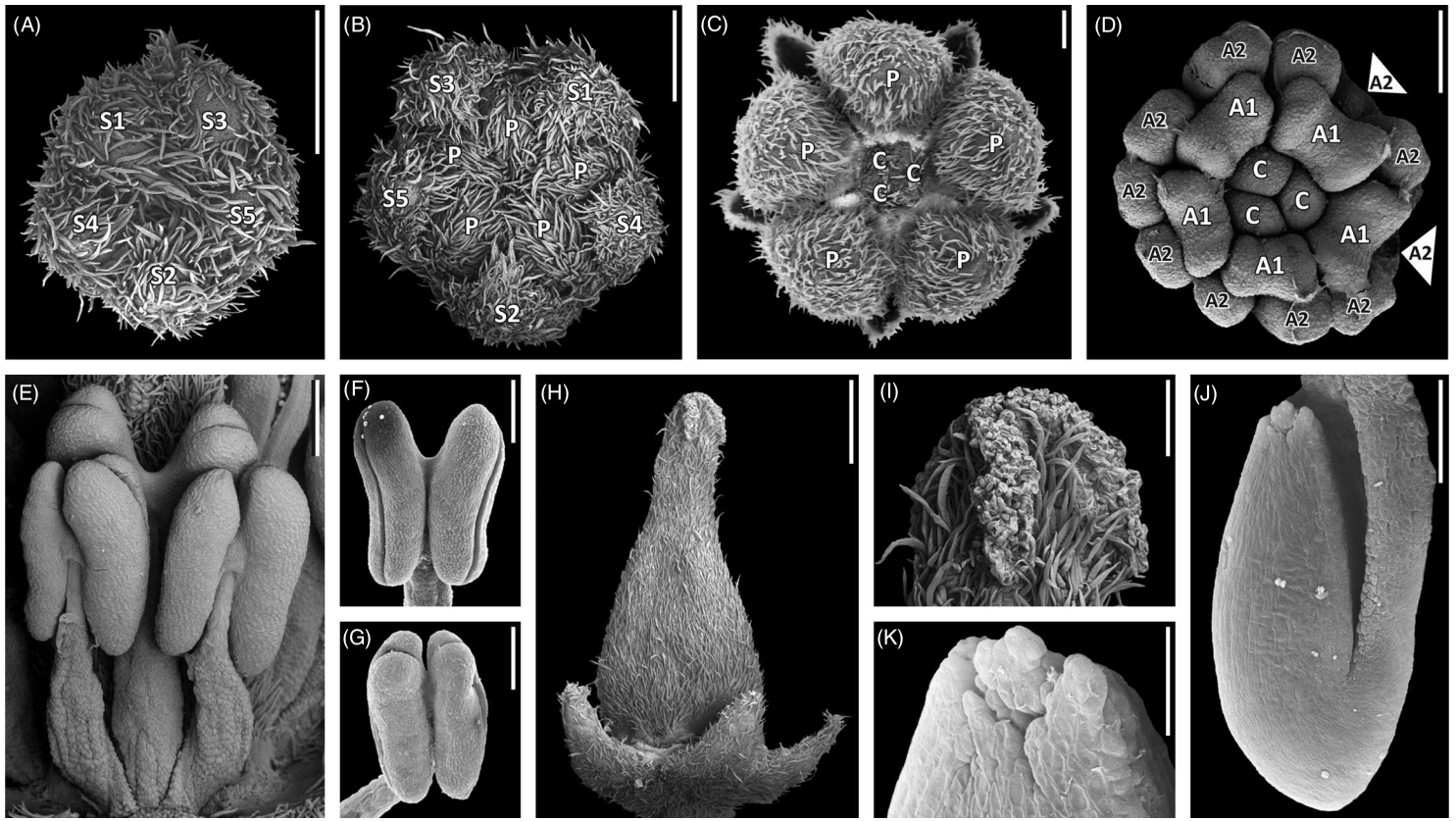

Fig 8.8 *Nitraria retusa*. Flower buds and floral organs. (A) Apical view of young closed bud showing the valvate sepal lobes (S1–S5). (B) Apical view of older bud showing separating sepals and valvate petals [P], carpels [c]. (C) Bud with expanding petals, carpels [c] visible. (D) Sepals and petals removed. Fifteen stamens surrounding three carpel tips, positions of missing antepetalous stamens (one of them not developed, one removed) indicated with arrowheads. (E) Dorsal view of a stamen triplet. (F), (G) Ventral view of anther. (F) Antesepalous stamen. (G) Antepetalous stamen. (H) Lateral view of anthetic gynoecium, petals and stamens removed. (I) Lateral view of stigmatic head, with two stigmatic crests. (J) Ovule, lateral view. (K) Detail of micropyle. Scale bars: (H) = 2 mm; (D), (E), (F), (G) = 1 mm; (A), (B), (C), (I), (J) = 500 μm; (K) = 200 μm.

shoulders (Fig 8.8F, G). Between androecium and gynoecium in the petal radii there is a small lobed nectary disc with five depressions (Figs 8.6E, 8.7L).

The gynoecium is syncarpous, and commonly trimerous. Sometimes it is dimerous and then disymmetric, or one of the three carpels is sterile and the gynoecium appears monosymmetric (Figs 8.6A–D, 8.7, 8.8C, D). The superior ovary is cylindrical and the style is conical and slightly swollen distally. The uppermost part of the gynoecium is apocarpous for a short distance, but with the carpels postgenitally united; the carpels are flat in this zone. Along the carpel margins, stigmatic tissue is decurrent for a short distance as three wavy lines along the style forming a short stigmatic head; stigmatic tissue has unicellular, slightly secretory papillae (Figs 8.7A–C, 8.8H, I, 8.9C). These three lines form three partial external compita (Figs 8.7, 8.8H, I, 8.9C). The gynoecium is of angiospermy type 3 or 4 (Fig 8.7).

The syncarpous zone encompasses the ovary and a large part of the style. The ovary is synascidiate for most of its length (Fig 8.7A, H–L). In the symplicate zone in the style the inner morphological surfaces of the carpels are partly contiguous, but not postgenitally united, thus forming a narrow stylar canal (Figs 8.7D–F, 8.9D). Only in the lowermost part (in the ovary) are they postgenitally united (Fig 8.7G, H). Below the stigmas, the PTTTs differentiate along the ventral side of the carpels. They consist of the epidermis, which is papillate, and fills the stylar canal with secretion (Fig 8.9D); the papillae point downward (Fig 8.7A–H).

The internal compitum is continuous with the external one and extends down to the transition between the symplicate and the synascidiate zone of the ovary (Fig 8.7A–H). At the transition between the synascidiate and the symplicate zones, only one of the two margins of each carpel differentiates into an axile lateral placenta and bears a single ovule. Sometimes a locule is empty at anthesis (Fig 8.7A, G–J). The ovule is inserted apically. It is long and cylindrical and fills the locule at anthesis (Figs 8.7A, H–J, 8.8J). It is anatropous, syntropous, crassinucellar and bitegmic (Figs 8.7A, H–J, 8.8J). The inner integument is 2–3 cell layers thick and is shorter than the outer one, which is 3–4 cell layers thick. As the placenta is lateral, the ovule has a somewhat oblique position in the locule (Fig 8.7A, H–J). Although the ovule is anatropous, the outer integument is relatively well developed on the concave side of the ovule (Fig 8.8J). Above the nucellus the inner integument is thicker than the outer one. The endostome is slit-shaped, whereas the exostome is closed by irregular lobes (Figs 8.7A, 8.8J, K). Both integuments together form a long and S-shaped micropyle (Fig 8.7A).

Anatomy and histology

The sepals have three main vascular traces, and there are up to seven branches formed by successive lateral branching (Fig 8.6A–E). The petals and stamens have a single vascular trace (Fig 8.6A–E). In petals, up to ten vascular bundles may be present (Fig 8.6A–D). In carpels the median dorsal vasculature forms a more or less

continuous band throughout the length of the gynoecium (Figs 8.6A–D, 8.7B–K). The lateral bundles form 'inverted' (xylem peripheral and phloem central) synlateral bundles in the septa of the ovary and each connects to a placenta (Fig 8.7E–H). In the synascidiate zone they become united in the floral centre, from where they extend downwards into the floral base (Figs 8.6E, 8.7I–L).

The perianth and gynoecium are covered by an indumentum of lignified unicellular hairs orientated towards the tips of the organs. Tanniferous cells are present in the hypodermis and mesophyll of almost all floral organs, including the ovule outer integument, and they are only lacking in stamens (Fig 8.9C, D, F, G). Special mucilage cells (for term, see Matthews and Endress, 2006) are present in all floral organs, in the stamen filaments and anther connectives, and in the gynoecium in the style and outer integument of the ovules (Fig 8.9F, G). They are lacking in the epidermis, but occur in the mesophyll (Fig 8.9F). They are differentiated successively from the outer to the inner organs during floral development, and from tip to base in petals and stamens.

8.3.3 *Tetradiclis tenella*

Morphology

Herbarium material does not allow for a description as extensive as for the other two species. The flowers are very small (less than 5 mm across), morphologically bisexual, tetramerous (also in the gynoecium), with a double perianth and haplostemonous (Fig 8.9H). The sepals appear free for most of their length; their aestivation is imbricate (decussate) (Eichler, 1878). The petals are free. In bud their aestivation is open at the base and imbricate further up. They become longer than the sepals during development and protect the inner organs up to anthesis. At anthesis, the perianth is wide open and the inner reproductive organs are entirely exposed.

The stamens are antesepalous and free. They have a broad filament base, which narrows further up. The transition from filament to anther is slightly constricted. The anthers are X-shaped, dorsally basifixed and likely versatile. The dorsal side of the thecae is slightly larger and longer than the ventral one. The anthers are slightly introrse. Each theca has a longitudinal dehiscence line, which encompasses its upper and lower shoulder. It is unclear whether a nectary is present between stamens and gynoecium.

The gynoecium is syncarpous and polysymmetric and has a superior ovary. The carpels are antepetalous (Fig 8.9H). The ovary is short and squared (Fig 8.9E, H, I). It gives the impression of being apocarpous because the carpel dorsal sides are strongly bulged and have deep longitudinal furrows running between them (Fig 8.9H, I). However, there is a single gynobasic, squared style (Fig 8.9E). Only the upper part of the style is apocarpous for a short distance, but the carpels are

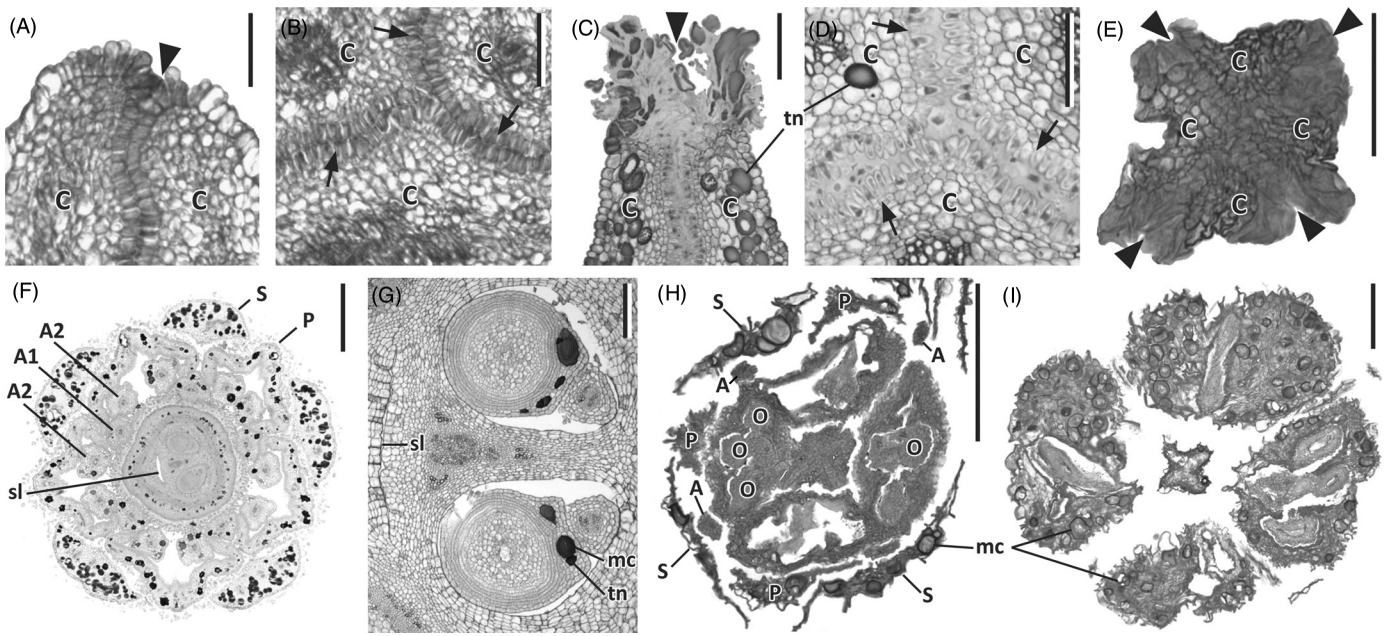

Fig 8.9 Nitrariaceae. Flower buds, and anthetic and postanthetic flowers, transverse sections. Signatures: Sepal [S], petal [P], antesepalous stamen [A1], antepetalous stamen [A2], carpel [C], ovule [O], sterile locule [sl], tanniferous cells [tn] are blue, special mucilage cells [mc] are pink to red. Arrowheads point to stigma and partial compitum, arrows point to carpel morphological surfaces covered with one cell-layered PTTT. (A)–(E) Styles and stigmas. (A), (B) *Peganum harmala*. (C), (D) *Nitraria retusa*. (E) *Tetradiclis tenella*. (F), (G) *Nitraria retusa*. (F) Preanthetic floral bud. (G) Anthetic gynoecium, with two locules with a single ovule and a sterile locule lacking an ovule. (H), (I) *Tetradiclis tenella*. (H) Anthetic flower. (I) Postanthetic flower. Scale bars: (F), (G), (H), (I) = 250 µm; (A), (B), (C), (D) = 100 µm; E = 50 µm. For colour illustration see plate section.

postgenitally connected in this zone and form a stigmatic head. Here the carpels are flat and their margins (flanks) are decurrent as four wavy stigmatic lines with unicellular papillae, which form four partial compita (Fig 8.9E). The gynoecium is of angiospermy type 4. In the syncarpous region, the synascidiate zone appears only short. The bulged part of the ovary forms an apical septum which extends almost down to the floor of the ovary. Below the stigmas, the PTTTs differentiate along the ventral side of the carpels; they are one cell layer thick and may be secretory (Fig 8.9E). The morphological surfaces of the carpel ventral sides are contiguous for the entire length of the style and form an internal compitum (Fig 8.9E, H, I), which is continuous with the external one and extends down to the base of the symplicate zone in the ovary. Because the synascidiate zone is so short, the two lateral placentae of each carpel each appear to be located at the base of each locule. The placentae are erect and each bears three (sometimes fewer) ovules. The ovules fill the locule at anthesis. The ovules are anatropous, syntropous, crassinucellar and bitegmic. Both integuments are 2–3 cell layers thick. The outer integument is longer than the inner and is hood-shaped, whereas the inner integument completely surrounds the nucellus. Both integuments are thickened above the nucellus and together form the micropyle, which faces the base of the funicle.

Histology

Special mucilage cells (for term, see Matthews and Endress, 2006) are present in sepals, petals and gynoecium (Fig 8.9H, I). Because of the poor preservation of the herbarium material used, it is unclear whether they are in the epidermal or subepidermal or even deeper cell layers of the perianth organs. During floral development these cells appear successively, from the sepals to the ovules. In the gynoecium they are present only after anthesis and last in the outer integument of the developing seeds (compare Fig 8.9H and I).

8.4 Discussion

8.4.1 Flower morphology, merism and symmetry

Flower size is diverse: large in *Peganum* (2 to 4 cm), small in *Nitraria* (*c.* 1 cm) and tiny in *Tetradiclis* (<5 mm). Flowers are (at least morphologically) bisexual and probably entomophilous in all three genera, although nectaries have been unambiguously reported only in *Nitraria* and *Peganum* (this study; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996; see discussion on Nectary). Flowers with a reduced number or incomplete development of organs of one of the two sexes have occasionally been observed both in *Nitraria* and in *Peganum*, and with reduction in ovule number per locule in *Tetradiclis* (this study; Baillon, 1873; Engler, 1931; Noble and Whalley,

1978). Partial functional unisexuality has only been clearly reported in *Nitraria* (Noble and Whalley, 1978). For *Peganum* and *Tetradiclis* it is unknown whether there are functionally unisexual flowers.

Floral *Bauplan* and merism are more similar between *Nitraria* and *Peganum* than between either of them and *Tetradiclis*. In *Tetradiclis*, the flowers are always completely isomerous (and therefore completely polysymmetric), independently of whether they are tetramerous or (more rarely) trimerous, and they have a single whorl of (antesepalous) stamens and antepetalous carpels (this study; Engler, 1931). In contrast, in *Nitraria* and in *Peganum*, the flowers have a pentamerous perianth and androecium, with fifteen stamens and a trimerous gynoecium (this study; Payer, 1857; Baillon, 1873; Eichler, 1878; Engler, 1931; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996; Hussein et al., 2009). Most notably, in both genera the 15 stamens are not arranged in two whorls as described by some earlier authors, but they form five triplets with a primary antesepalous stamen flanked by two secondary stamens. This can be interpreted as a specialized haplostemonous pattern. In addition, because of the difference in merism between the gynoecium and the outer organs, the flowers in *Nitraria* and *Peganum* exhibit an (inconspicuous) monosymmetry (in *Peganum* along the median plane; Eichler, 1878). The variation in organ number observed in both genera is also similar. In *Nitraria* the number of antepetalous stamens may vary and the androecium may even be reduced to a single whorl of five antesepalous stamens (Baillon, 1873; Engler, 1931). More commonly, one of the three carpels is sterile and lacks an ovule in the locule, or it may even be so reduced that the gynoecium appears dimerous (this study; Baillon, 1873); Baillon (1873) also mentions cases with up to six carpels. In *Peganum*, the perianth and androecium may be tetramerous (Engler, 1931). Independently of the other organ whorls, the gynoecium may be reduced to two carpels or, more rarely, have four carpels, or even five (Baillon, 1873; Engler, 1931; Shukla, 1955; Li and Tu, 1994). It has been suggested that the trimerous gynoecium in *Peganum* may be derived from a pentamerous one by reduction of two carpels because of the pentagonal shape of the syncarpous gynoecium ring primordium in early stages of development (Ronse De Craene et al., 1996). We also observed that the shape of the trimerous gynoecium may not be as triangular as would be expected, but we did not observe the initiation or the development of more than three carpels. Thus, because of the phylogenetic position of *Tetradiclis*, the idea cannot be ruled out that the flowers of *Peganum* are derived from isomerous, fully pentamerous ancestral forms. It is more likely, however, that the pentagonal shape of the gynoecium is superimposed by the pentamerous androecium as in analogous cases of other families discussed by Endress (2006, 2008).

Small-sized entomophilous flowers with a nectary are common in Sapindales and other rosids (see discussion on Nectary). Morphologically bisexual flowers

with one of the two sexes more or less reduced or aborted, and thus functionally unisexual (as partly in *Nitraria*), have also been reported in almost all other sapindalean families, except for Biebersteiniaceae about which little is known (i.e. Anacardiaceae, Burseraceae, Kirkiaceae, Meliaceae, Rutaceae and Simaroubaceae; see Bachelier and Endress, 2008, 2009). In Sapindales, flowers are commonly isomerous and polysymmetric as in *Tetradiclis*. Flowers in which the gynoecium is not isomerous with the other floral whorls exhibit a kind of inconspicuous (oblique) monosymmetry (such as in Nitrariaceae, except for *Tetradiclis*). Isomerous and polysymmetric flowers are pentamerous in Biebersteiniaceae (Reiche, 1889), tetramerous in Kirkiaceae (Stannard, 1981; Bachelier and Endress, 2008), and can variously have three, four or five organs per whorl in Anacardiaceae, Burseraceae, Meliaceae, Rutaceae or Simaroubaceae (Bachelier and Endress, 2009). Such inconspicuously monosymmetric flowers with an oblique symmetry also occur in Anacardiaceae and Burseraceae (Bachelier and Endress, 2009). However, pronounced monosymmetry with unequal differentiation of certain floral sectors is less common and not present in Nitrariaceae. It is known from Sapindaceae, some Rutaceae and exceptionally in Meliaceae (Ronse De Craene et al., 2000; Weckerle and Rutishauser, 2003, 2005; Endress and Matthews, 2006; Ronse De Craene and Haston, 2006; Endress, accepted).

Perianth

The perianth has a calyx and corolla in all three genera. In *Nitraria* and *Peganum*, the sepals are initiated in a clockwise or anticlockwise spiral sequence. In contrast, the five petals are initiated more or less simultaneously. In both genera, the second sepal is in median adaxial position as is common in core eudicots (this study; Payer, 1857; Ronse De Craene, 2010; for *Nitraria*, also Baillon, 1873; for *Peganum*, also Eichler, 1878). The abaxial petal between sepals 1 and 3 (= petal 1) is slightly larger than the others at first, in both genera (this study; Ronse De Craene et al., 1996). In *Tetradiclis* the first pair of sepals is in the median plane (Eichler, 1878).

The sepals are free in early stages of development in both genera, but later they appear (congenitally) united basally in *Nitraria*. Sepal aestivation changes during development: at first the margins become contiguous and may overlap later, which results in valvate or imbricate (quincuncial) aestivation. This may account for the different descriptions of sepal aestivation in the literature: valvate in *Nitraria* and *Peganum* (Baillon, 1873; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963), or imbricate in *Nitraria* (Baillon, 1873; Nair and Nathawat, 1958; Liu and Zhou, 2008) and in *Peganum* (Payer, 1857; Baillon, 1873; Ronse De Craene et al., 1996). In *Tetradiclis* sepal aestivation is imbricate (decussate) (Eichler, 1878).

The petals are free in all genera throughout development. Their aestivation is somewhat open at the base, but induplicate-valvate further up in *Nitraria* (and

the petal tips are strongly bent inwards), whereas it is imbricate in *Tetradiclis* and *Peganum* (contort in *Peganum*, cochlear in *Tetradiclis*) (this study; Payer, 1857; Baillon, 1873; Eichler, 1878; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). In all three genera, the aestivation of the sepals becomes open during development; the petals become longer than the sepals, and thus, protect the inner floral organs up to anthesis (this study; Ronse De Craene and Smets, 1991). Postgenital coherence between petals was found in *Nitraria* and *Peganum* (this study), but is unknown in *Tetradiclis*. After anthesis, the calyx is persistent in all genera, while the corolla and androecium are persistent only in *Tetradiclis*, but caducous in *Nitraria* and *Peganum* (this study; Baillon, 1873; Noble and Whalley, 1978; Ronse De Craene and Smets, 1991; Hamzaoğlu et al., 2005; Liu and Zhou, 2008).

Flowers with a pentamerous calyx, in which the sepals are initiated in a quincuncial spiral sequence with the second sepal in abaxial (posterior) position, such as in *Nitraria* and *Peganum*, are common in Sapindales and other rosids (Ronse De Craene, 2010). That the corolla protects the inner floral organs up to anthesis, with a postgenital coherence between the petals (formed by the papillate epidermis or interlocking hairs), also occurs in other Sapindales (Bachelier and Endress, 2009; for details, see Ronse De Craene and Haston, 2006, p. 468), and in other rosids, especially in malvids (Endress and Matthews, 2006; Matthews and Endress, 2006; Endress, 2010).

Androecium

The androecium is the most puzzling and therefore most frequently discussed part of the flowers in Nitrariaceae. It has one stamen whorl in *Tetradiclis*, but in *Nitraria* and *Peganum*, it seems to have two whorls. In addition, the androecium in *Nitraria* and *Peganum* does not follow the rule of organ alternation (as in diplostemonous flowers), in that the antesepalous whorl of stamens appears to be the inner one, and the antepetalous the outer one. This condition has generally been referred to as obdiplostemony (Payer, 1857; Beille, 1902; Saunders, 1937; Eckert, 1966; Ronse De Craene and Smets, 1991; Ronse De Craene and Smets, 1995; Ronse De Craene et al., 1996). However, what is most unusual in Nitrariaceae is that the apparent antepetalous whorl commonly comprises ten stamens arranged in antepetalous pairs (Payer, 1857; Eckert, 1966; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). The structure of the androecium and the origin of the antepetalous stamens in *Nitraria* and *Peganum* have been differently interpreted. Earlier comparative studies interpreted the androecium of *Nitraria* as derived from a haplostemonous pattern and thus each antesepalous stamen plus the two closest antepetalous stamens should be seen as a triplet of antesepalous stamens (Baillon, 1873; Ronse De Craene and Smets, 1991). As an exception, haplostemonous flowers with only five antesepalous stamens were

reported in *Nitraria* (Baillon, 1873). In contrast, in *Peganum*, the androecium was interpreted as derived from a diplostemonous androecium pattern, with the antepetalous stamen pairs having doubled antepetalous positions (Payer, 1857; Beille, 1902; Ronse De Craene et al., 1996). Only Nair and Nathawat (1958) suggested that the antepetalous stamens in *Peganum* belong to antesepalous triplets as in *Nitraria*, but only based on mature stages. In both *Nitraria* and *Peganum* the first stamen primordia to appear are antesepalous (this study; Payer, 1857; Beille, 1902; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). Our developmental studies show that the antepetalous stamens of *Peganum* arise close to the antesepalous ones. The further development of the androecium in *Peganum* is indeed very similar to that in *Nitraria*, contrary to earlier claims of a difference. Therefore the origin of the antepetalous stamens is very likely the same in *Nitraria* and *Peganum*, and probably derived from a haplostemonous pattern as found in *Tetradiclis*. Ronse De Craene et al. (1996) erroneously interpreted the antepetalous stamens as double on the evidence that the antepetalous stamens have a separate vascular connection from the antesepalous stamen and developmental evidence that the antepetalous primordia converge towards each other and appear to be similar in size, contrary to stamens belonging to a triplet. However, pressure by the petals may have caused differences in the development of individual stamens (Fig 8.5K).

Stamen structure is similar in all three genera. The filament is broad at the base and rounder further up. The filament tip is constricted and the anther, which is basifixed and sagittate or X-shaped, is shorter than the filament. The filament base thickens at anthesis in both *Nitraria* and *Peganum*, and its margins become more or less flattened (this study; Payer, 1857; Nair and Nathawat, 1958). Only in *Peganum* are the anthers apiculate. A notable difference in our material is that at anthesis the antepetalous stamens are longer than the antesepalous ones in *Peganum*, but the other way around in *Nitraria*.

In Sapindales, flowers are commonly diplostemonous, whereas haplostemonous flowers are much less common. Nevertheless, they have a scattered occurrence in almost all families, except for the unigeneric Biebersteiniaceae (Bachelier and Endress, 2008, 2009). In addition, when the flowers are haplostemonous, only the antesepalous stamens are present as in *Tetradiclis*. This condition may be the result of a reduction of the antepetalous stamens (see Bachelier and Endress, 2009). In contrast, the development of polyandrous flowers with more stamens than the expected number based on floral merism, is much rarer in Sapindales, and has been reported in a very few genera such as *Gluta*, *Sclerocarya* and *Sorindeia*, in Anacardiaceae (Ding Hou, 1978; von Teichman and Robbertse, 1986; Breteler, 2003), *Canarium* in Burseraceae (Lam, 1932), *Chisocheton*, *Clemensia, Turraea* and *Vavaea* in Meliaceae (Harms, 1940), *Mannia* in Simaroubaceae (Engler, 1931),

Deinbollia in Sapindaceae (Eichler, 1878) and *Citrus* and *Aegle* in Rutaceae (Leins, 1967; Lord and Eckard, 1985). In such cases, there are commonly more than two whorls of stamens. Triplets of antesepalous stamens as in *Nitraria* and *Peganum* are, to our knowledge, not known in any other sapindalean family. Among other rosids they are known from Geraniaceae in Geraniales (*Hypseocharis*, *Monsonia*, *Sarcocaulon*, Payer, 1857; Saunders, 1937; Narayana and Arora, 1963; Rama Devi, 1991; Ronse De Craene and Smets, 1996), Elaeocarpaceae in Oxalidales (Matthews and Endress, 2002) and Ixonanthaceae in Malpighiales (Narayana and Rao, 1966; Ronse De Craene and Smets, 1996). In addition, antepetalous stamens are in general smaller than antesepalous ones across the eudicots (e.g. Bachelier and Endress, 2009). Thus, longer antepetalous stamens, such as in *Peganum*, are unusual and have been reported elsewhere in Sapindales only in *Protium* in Burseraceae (Daly, 1992; Bachelier and Endress, 2009).

Stamens such as those in Nitrariaceae, with a sagittate and basifixed anther, and longitudinal dehiscence slits extending for the entire length of the thecae, are common in other rosids in general (Endress and Stumpf, 1991). The exact phylogenetic position of Nitrariaceae in Sapindales is not yet completely clear and it is therefore uncertain whether the unusual androecium structure of *Nitraria* and *Peganum* is a synapomorphy for the family, with a secondary reduction in *Tetradiclis*, or whether it is a convergence derived from a haplostemonous pattern in both *Nitraria* and *Peganum*. However, it is a good feature to support the monophyly of Nitrariaceae.

Nectary

In *Nitraria* and *Peganum*, floral nectaries are present between the androecium and the gynoecium (this study; Nair and Nathawat, 1958). They have been described as a more or less developed intrastaminal lobed disc in *Peganum* (Baillon, 1873; Kapil and Ahluwalia, 1963; Ronse De Craene et al., 1996) and as antepetalous pits in *Nitraria* (Ronse De Craene and Smets, 1991). However, we found that the nectaries are similar in both genera in consisting of five depressions in the petal radii. Nectaries have not been reported as yet in *Tetradiclis*.

An intrastaminal floral nectary is common in Sapindales and most other rosids (Ronse De Craene and Haston, 2006). However, the presence of conspicuous nectariferous depressions in the petal radii, as in *Peganum* and *Nitraria*, is unusual. This feature could thus be another synapomorphy for Nitrariaceae.

Gynoecium

The gynoecium is syncarpous in all Nitrariaceae (this study; Baillon, 1873; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963; Ronse De Craene and Smets, 1991; Ronse De Craene et al., 1996). The synascidiate zone is present in variable

proportions (longest in *Peganum*, shortest in *Tetradiclis*). The symplicate zone extends to the base of the stigmas. The stigmatic zone is apocarpous, but with the carpels contiguous or postgenitally connected into a stigmatic head. The carpels are flat in this zone and their margins form three decurrent stigmatic lines (if the gynoecium is tricarpellate). In *Tetradiclis* and *Peganum* the dorsal parts of the carpels are bulged in the ovary, extremely so in *Tetradiclis*, which results in a morphologically very short ovary, an apical septum and therefore also a very short synascidiate zone. In *Peganum* and *Tetradiclis* the base of the style is hidden by the dorsal bulges of the ovary. However, in all three genera, the style is (morphologically) apical and it is rounder at the base than further up, and distally, the style and stigmatic head are conspicuously angled, with as many angles as there are carpels (this study; Baillon, 1873; Kapil and Ahluwalia, 1963). The stigmatic lines along the edges of the stigmatic head are covered with unicellular papillae in all three genera. They form an external compitum which is continuous with the internal compitum formed by the contiguous ventral sides of the carpels. In *Nitraria*, the gynoecium is of angiospermy type 3 or 4, and the style is hollow in transverse section, with as many slits as there are carpels, and it is filled with secretion. In contrast, in *Peganum*, the gynoecium is of angiospermy type 4 and the ventral sides of the carpels are tightly appressed to each other and postgenitally connected. In *Tetradiclis*, the inner part of the style appears as in *Peganum* at anthesis, but later becomes hollow as in *Nitraria* and is filled with secretion or remains of the former PTTT (Fig 8.9I).

In Sapindales, the gynoecium is commonly syncarpous (Bachelier and Endress, 2009), especially in the putative basal groups (Biebersteiniaceae, Nitrariaceae). Also, the presence of a stigmatic head formed by the free, but postgenitally united, carpel tips is relatively more widespread in Sapindales (Endress et al., 1983; Endress and Matthews, 2006; Bachelier and Endress, 2009) and malvids in general than in other eudicots (Endress, 2010). However, in contrast to Nitrariaceae, in other Sapindales the receptive surface is not restricted to the carpel margins. Stigmatic lines decurrent along the edges of an angled stigmatic head may thus be a synapomorphy for Nitrariaceae. In addition, continuity between the external compitum formed by the stigmatic head and an internal compitum, such as in Nitrariaceae, is probably not common in Sapindales. It has been poorly studied, but it occurs in core Burseraceae (*Canarium*, Bachelier and Endress, 2009), and may also be present in some Rutaceae (Ramp, 1988). Unicellular stigmatic papillae, such as in Nitrariaceae, are common in Sapindales. Only in the clade of Kirkiaceae and Anacardiaceae + Burseraceae are the papillae multicellular and uniseriate (Bachelier and Endress, 2009). The presence of a gynophore, such as in *Peganum*, is also common in Sapindales and other malvids (Endress and Matthews, 2006; Endress, 2010). In all three genera the placentae are lateral, even in *Nitraria*, which has only one ovule per carpel.

Ovules

The ovules are crassinucellar and bitegmic (this study; Mauritzon, 1934; Kapil and Ahluwalia, 1963; Kamelina, 1994; Li and Tu, 1994). Both integuments contribute to the formation of the micropyle. In *Malacocarpus* the micropyle is zigzag-shaped (Kamelina, 1985). In *Nitraria* and *Peganum* the outer integument is slightly thicker than the inner one (this study; Mauritzon, 1934; Kapil and Ahluwalia, 1963; Li and Tu, 1994). In *Tetradiclis*, the integuments are equally thick (this study; Kamelina, 1994). The ovules are anatropous (Payer, 1857; Baillon, 1873; Nair and Nathawat, 1958; Kapil and Ahluwalia, 1963; Kamelina, 1994). However, in *Nitraria* and *Tetradiclis* the outer integument is relatively long on the concave side of the ovules, in contrast to those in *Peganum* and *Malacocarpus* (this study; Kamelina, 1985, 1994). It would be interesting to know whether this long part of the outer integument develops early or only appears late in development, as an adjustment to the available space in *Nitraria* and *Tetradiclis*. The direction of the curvature is syntropous in all genera. Also in all genera, the funicle is relatively long, especially in *Nitraria* and in *Tetradiclis* (Fig 8.10; this study; Kamelina, 1985, 1994).

Ovules are most commonly anatropous, bitegmic and crassinucellar in rosids. In Sapindales and other malvids there is a tendency for the inner integument to be thicker than the outer (Endress and Matthews, 2006; Endress, 2010), a feature not present in Nitrariaceae. A long funicle, such as in Nitrariaceae, is especially frequent in Anacardiaceae. Interestingly, both families have syntropous ovules and

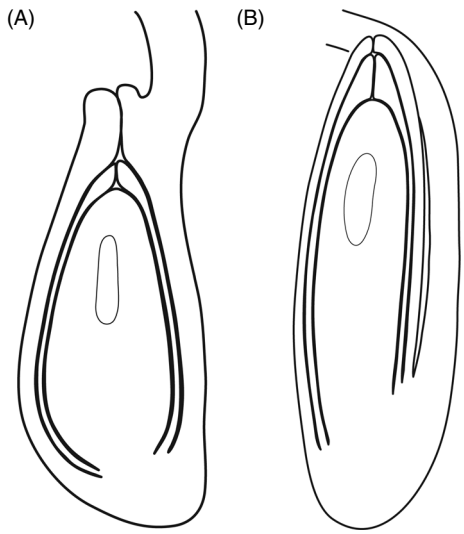

Fig 8.10 Ovules in Nitrariaceae. Schematic median longitudinal sections. (A) *Peganum harmala*. (B) *Nitraria retusa*.

a long funicle may thus be functionally linked to the direction of ovule curvature (Bachelier and Endress, 2009).

8.4.2 Anatomy

In *Nitraria* and *Peganum*, the sepals have three vascular traces and the petals one vascular trace, as is common in eudicots. The dorsal vasculature of the carpels appears as broad reticulate bands of anastomosing bundles. The synlateral bundles are inverted and have the xylem in peripheral position (for *Nitraria*, see also Shukla, 1955; Nair and Nathawat, 1958; for *Peganum*, see also Beille, 1902).

A diffuse or weakly differentiated carpel dorsal vasculature, such as in *Nitraria* and *Peganum*, is a feature that appears to be relatively pronounced in Sapindales (Ronse De Craene and Haston, 2006; Bachelier and Endress, 2008, 2009). Also, inverted synlateral vascular bundles have been reported in Sapindales, such as in some Burseraceae (Stevens, 2001 onwards; Bachelier and Endress, 2009).

8.4.3 Histology

Tanniferous cells are present in *Nitraria* and are lacking in *Tetradiclis* and *Peganum*. Special mucilage cells (for term, see Matthews and Endress, 2006) are present in the hypodermis and mesophyll of all floral organs in *Nitraria* and *Tetradiclis* (this study; Kamelina, 1994). In both genera their differentiation proceeds centripetally, in parallel to the differentiation of the organs. They are already present in all organs at anthesis in *Nitraria*, whereas in *Tetradiclis*, they become conspicuous only after anthesis in the wall of the developing fruit, and even later in the seed coat (this study; Kamelina, 1994). In addition, in flowers of *Nitraria*, differentiation also clearly proceeds from the tip of the organs downwards in petals and anthers, and special mucilage cells may thus also be present in the ovary after anthesis. Therefore, the idea cannot be ruled out that mucilage cells also develop in the reproductive organs of *Peganum* or *Malacocarpus* after anthesis, since they are present in their leaves, as in *Nitraria* and *Tetradiclis* (Metcalfe and Chalk, 1950; Sheahan and Cutler, 1993). Special mucilage cells occur in many other orders of eudicots (but sometimes only in leaves) and their presence is more or less consistent at the familial level (survey in Matthews and Endress, 2006).

8.4.4 Floral structure and systematics

Features supporting the exclusion of *Nitraria, Tetradiclis*, and *Peganum* from Zygophyllaceae

- Leaves with spiral phyllotaxis (versus opposite in Zygophyllaceae; El Hadidi, 1975; Liu and Zhou, 2008)
- Flowers with pentamerous perianth and androecium but trimerous gynoecium, or flower isomerous and tetramerous but haplostemonous (versus isomerous,

pentamerous and diplostemonous in Zygophyllaceae; this study; Engler, 1931; Nair and Nathawat, 1958)

- Petal development not delayed and corolla protecting the inner organs in bud (versus delayed in most Zygophyllaceae; Ronse De Craene, pers. obs. in *Fagonia*, *Zygophyllum* and *Tribulus* – not delayed in *Balanites*; this study)
- Ovules crassinucellar and without endothelium (versus sometimes weakly crassinucellar [for definition see Endress, 2010], and almost, always with endothelium, even if crassinucellar, in Zygophyllaceae; this study; Mauritzon, 1934; Nair and Gupta, 1961; Kapil and Ahluwalia, 1963; Masand, 1963; Kamelina, 1985; Li and Tu, 1994)
- Ovules syntropous (versus antitropous in Zygophyllaceae; this study; Nair and Jain, 1956; Narayana and Prakasa Rao, 1962, 1963; Li and Tu, 1994)
- Micropyle formed by both integuments (versus only inner integument in Zygophyllaceae, but formed by both integuments also in *Balanites* and *Seetzenia*; this study; Kamelina, 1985)
- Funicle long and outer integument unusually well developed on the concave side of the ovule in *Nitraria* and *Tetradiclis* (not well developed at anthesis in *Peganum* and *Malacocarpus*) (versus short and not well developed in Zygophyllaceae; this study; Kamelina, 1985, 1994).

Features shared by *Nitraria*, *Tetradiclis*, and *Peganum* supporting their inclusion in Sapindales

- Carpel tips free and flat, but postgenitally connected forming a stigmatic head with external compitum and stigmas decurrent along the carpel margins (*Kallstroemia* in Zygophyllaceae, which has a superficially similar gynoecium tip, whose structure is unknown in detail, also needs to be analysed and compared to Nitrariaceae)
- Ovary bulged dorsally so that the style appears 'gynobasic' in *Peganum* and *Tetradiclis* (not in *Nitraria*)
- Nectaries intrastaminal (perhaps lacking in *Tetradiclis*)
- Special mucilage cells present in floral organs.

Potential synapomorphies for a broad circumscription of Nitrariaceae (including *Nitraria*, *Tetradiclis* and *Peganum* + *Malacocarpus*) (features rather uncommon in other sapindalean families)

- Flowers haplostemonous, or derived from a haplostemonous pattern (antesepalous triplets)

- Stigmatic head angled, with as many angles as there are carpels
- PTTT one cell layer thick
- Compitum extending from stigmas to placentae, and external and internal compitum continuous
- Ovules syntropous with a long funicle
- Nectaries forming antepetalous depressions (perhaps lacking in *Tetradiclis*).

Potential apomorphies for *Nitraria* if considered as a monogeneric family (excluding *Tetradiclis* and *Peganum* + *Malacocarpus*)

- Style hollow filled with secretion and unicellular papillate PTTT
- Ovary without dorsal bulges and with apical placenta
- Ovule one per carpel.

Potential apomorphies for the clade of *Tetradiclis* + *Peganum* (excluding *Nitraria*)

- Angiospermy of type 4
- Carpels with conspicuous dorsal bulges and placentae basal or at mid-length
- Ovules more than one per carpel (and more than one seed per fruit)
- Funicle much longer than the remainder of the ovule.

8.5 Conclusions

This study shows the interdependence of 'modern' phylogenetic reconstructions and 'traditional' comparative approaches for the study of angiosperm systematics and evolution. Using the example of Nitrariaceae, we have demonstrated how the new light shed on the relationships by molecular-based methods has changed our understanding of the evolution of the flower structure and unusual androecium of Nitrariaceae as circumscribed now in APG (2009). In addition, this study shows that the exclusion of *Nitraria*, *Tetradiclis* and *Peganum* from Zygophyllaceae, and their inclusion in Sapindales, is well supported by floral structure. The morphological variation observed between the three genera of Nitrariaceae is reminiscent of that found in the clade of Kirkiaceae and Anacardiaceae + Burseraceae (Bachelier and Endress, 2008, 2009). In addition, the estimated age of divergence between these genera is similar to that estimated between these three sapindalean families (Muellner et al., 2007), and the divergence between their *rbc*L sequences is as extensive as that between other families of Sapindales (Sheahan and Chase, 1996). Therefore, this study also shows that the separation of the Nitrariaceae,

as circumscribed in APG (2009), into three families (e.g. Takhtajan, 2009), with Nitrariaceae being unigeneric, Tetradiclidaceae being monotypic and Peganaceae comprising two genera, of which *Malacocarpus* would be monotypic, could be supported. However, this study also shows that these three genera share features of the androecium and gynoecium which are unusual in other Sapindales and thus could as well be included in a single family. Additional molecular phylogenetic studies and comparative morphological studies of other families of Sapindales are thus urgently needed and we can only hope for more interdisciplinary collaboration in future to understand the systematics and evolution of Sapindales and angiosperms.

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